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Antifeedant activity and disruptive effects of novel chitin synthesis inhibitors on the food metabolism of last instar larvae of *Spodoptera littoralis* Boisd. (Noctuidae: Lepidoptera)

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ABSTRACT

The present study was conducted to investigate the antifeedant activity of three novel chitin synthesis inhibitors (CSIs), viz. Novaluron (Nova.), Cyromazine (Cyro.) and Diofenolan (Dio.), and their disruptive effects on the different nutritional parameters of the destructive phytophagous pest Spodoptera littoralis. Fresh clean castor bean leaves were treated with LC_{50} of each CSI and offered to the early last instar larvae for 24 hrs or 72 hrs. All CSIs exhibited considerably antifeedant activities against larvae. Higher activity was exhibited after longer feeding period. Nova. exhibited the strongest antifeedant activity followed by Dio. and Cyro.. The food consumption was remarkably reduced, regardless the CSI and the feeding period. The strongest reducing effect was exhibited by Nova. followed by Dio. and Cyro.. Treated larvae achieved significantly inhibited approximate digestibility. Nova. exhibited the most potent effect followed by Dio. and Cyro.. The efficiency of conversion of ingested food into biomass (ECI) of larvae was severely inhibited by the tested CSIs. After feeding for 72 hrs, an exceptional case was observed since Nova. Enhanced ECI of larvae. The efficiency of conversion of digested food into biomass (ECD) was considerably enhanced after feeding on treated leaves for 24 hrs. In contrast, feeding of larvae for 72 hrs resulted in serious reduction of ECD. Also, the assimilation rate, relative metabolic rate, relative weight gain and growth rate were significantly regressed by all CSIs. The most suppressing action was exerted by Nova.. On the other hand, frass output was enhanced by all CSIs whatever the feeding period on treated leaves. Nova. was the most powerful CSI to promote larvae for frass production.

Keywords: assimilation, biomass, Cyromazine, consumption, conversion, digestibility, diofenolan, faeces, growth, novaluron.

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INTRODUCTION

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is a polyphagous insect. Approximately112 plant species belonging to 44 families are reported as hosts of this pest in tropical and temperate zones of the old world [1] or 73 species recorded from Egypt [2]. In Egypt, this destructive phytophagous lepidopterous pest attacks cotton, various vegetables and field crops all over the year [3-6]. When large numbers of the pest are present complete crop loss is possible [7]. To control the attacks of *S. littoralis*, several types of insecticides have been used, including synthetic pyrethroids, organophosphates, and non-steroidal compounds [8].The extensive use of these insecticides has caused resistant insect strains to emerge making their control even more difficult [9-14]in addition to serious toxicological problems of the synthetic pesticides, such as increased costs, handling hazards, several adverse effects on food, soil, ground water and air as well as carcinogenic, teratogenic and great threats to both human and environmental health [15-19].

Owing to the socioeconomic importance of *S. littoralis*, the insect is subject to extensive research, much of which is focused on finding new ways to control it as a pest and to improve the effects of known pest control methods [20]. To overcome those problems of synthetic pesticides, it is necessary to seek safe, convenient, environmental and low-cost alternative pest control methods among which are the insect growth regulators (**IGRs**). They were developed to mimic, block or otherwise interact with the hormonal system of insects [21]. On the basis of the mode of action, IGRs are

grouped into three categories: juvenile hormones and their analogues (Juvenoids); ecdysone agonists or ecdysteroids; and chitin synthesis inhibitors (**CSIs**) or moult inhibitors [22,23]. **IGRs**, *viz.*, juvenoids, anti juvenoids, ecdysteroids and **CSIs** as well as other related compounds, have been reported to possess a specific activity spectrum with a novel mechanism not based on a neurotoxic action, like synthetic insecticides [24]. The use of **IGRs** in pest control is known as insect development inhibitors which inhibits or prevents normal metamorphosis of immature stages to the adult stage [25-27]. IGRs are "low risk" insecticides, which have a relatively minor detrimental effect on the environment and its inhabitants, rendering them important components in IPM programs [28].

Novaluron is a relatively new benzoylphenyl urea **CSI** with good activity against the Colorado potato beetle [29-32] and low mammalian toxicity [33,34]. **Novaluron** was found as an deteriorating effective CSI on survival and development [35] and adult performance of *S. littoralis* [36]**.** Its residues tend to dissipate with half-life of 2.08 days and the safe use of it on tomatoes, and possibly on other crops in Egypt was established [37]. **Cyromazine** is a triazine IGR used as alternative to insecticides and acaricides. It is used in veterinary medicine for the protection of animals, such as sheep and lamps, against flies [38]. As reported by many authors [39-44], **Cyromazine** exhibited various degrees of success for controlling different pests such as house flies and leafminers. It exhibited remarkable toxic and inhibitory effects on growth of *S. littoralis* [45]. **Diofenolan** is a CSI used for the control of several pests, such as lepidopterous species and scale insects [46-48], *Papilio demoleus* [49], *Musca domestica* [50-53], *Rhynchophorus ferrugineus* [54] and *Schistocerca gregaria* [55-58]. It did not affect the survival of beneficial parasitoids and predators of some pests such as *Chrysoperla carnea* [59].

Feeding and reproduction in insects are very closely related to nutritional factors, the qualitative and quantitative aspects of which have impact on the rate of growth, development and fecundity. Since the amount, rate and quality of food consumed by a larva influences its performance, growth rate, development time, final body weight and survival [60]. Therefore, an understanding of the nutritional indices in relation to the rate of ingestion, digestion assimilation and conversion by the growing larvae would be useful [61]. Also, reduction in feeding activity of insect may reduce normal development, weight gain, fecundity and increase mortality [62].

It is important to point out that some of the natural products or synthetic chemicals disrupt the hormonal balance in insects by inhibiting the growth, metamorphosis and reproduction while other chemicals affect the feeding behavior of the insects and inhibit feeding. As defined by some authors [63-66], antifeedant is a chemical that inhibits the feeding without killing the insect pest directly, while it remains near the treated foliage and dies through starvation. Some insecticides, IGRs and botanicals have been found as appetite inhibitors for insects. Because deterrence is the act of preventing a particular act or behavior from happening, these compounds and products can be described as food deterrents, phagodeterrents or antifeedants against insects.

In insects, the physiological events that are linked to food consumption and utilization appear to be controlled by neural, endocrine and secretagogue mechanisms [67,68]. Hormones produced by the brain neurosecretory cells, the corpora cardiaca and corpora allata also control the digestive enzyme production [69]. As for examples, in the last in star larvae of *Spodoptera mauritia*, feeding activity is maximum at high Juvenile hormone (JH) titer but when JH titer declines and the subsequent release of ecdysteroids, the feeding activity decreases[70,71]. Besides their lethal action on insect immature stages and sterility in sexually mature adults, **IGRs** also inhibit the **food consumption** and **growth** of individuals which survive after sublethal treatments [72]. **IGRs** are known to affect the digestion, utilization and other metabolic processes of ingested food [73]. However, the action of some juvenoids, ecdysteroids and CSIs on food consumption and utilization was investigated in various insect species [74-79]. Therefore, the **present work was conducted to investigate** the antifeedant activity and the disruptive effects of CSIs, *viz*., Novaluron, Cyromazine and Diofenolan, on food consumption and utilization in the last larval in star of *S. littoralis*.

MATERIALS AND METHODS

1. Experimental insect:

A sample of the Egyptian cotton leaf worm *Spodoptera littoralis* pupae was kindly obtained from the culture of susceptible strain maintained for several generations in Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt. In laboratory of Entomology, Faculty of Science, Al-Azhar University, Cairo, a

culture was reared under laboratory controlled conditions (27±2°C, 65±5% R.H., photoperiod 14 h L and 10 h D). Rearing procedure was carried out according to [80] and improved by [81]. Larvae were provided daily with fresh castor bean leaves *Ricinus communis*. The emerged adults were provided with 10% honey solution on a cotton wick as a food source. Moths were allowed to lay eggs on branches of *Neriumoleander*, then the egg patches were collected daily, and transferred into Petri dishes for another generation.

2. Larval treatments with CSIs:

Novaluron (Rimon, Pestanal®) [1-[chloro-4-(1,1,2-trifluoromethoxyethoxy) phenyl] -3- (2,6-difluorobenzoyl) urea] was purchased from Sigma-Aldrich Chemicals (https://www.sigmaaldrich.com), **Cyromazine** (Larvadex, Trigard, Vetrazin) [N-cyclopropyl-1, 3, 5-triazine-2, 4, 6-triamine] was purchased from Sigma-Aldrich Chemicals (https://www.sigmaaldrich.com) and **Diofenolan** (CGA 59205, Aware®)[2-ethyl-4-[(4-phenoxyphenoxy) methyl]-1,3-dioxolane] was obtained from Agricultural research center, laboratory of pesticides, Doqqi, Giza, Egypt.

Most of the total food consumption and growth usually occur during the penultimate and last larval instars and therefore performance values calculated for these instars tend to be representative of those calculated for the entire larval stage [61]. Therefore, the last (6^{th}) larval instar of *S. littoralis* was chosen in the present study. In a preliminary experiment,**LC_{50} values** of Novaluron, Cyromazine and Diofenolan were calculated, after treatment of last instar larvae of *S. littoralis*, as 2.71, 74.44 and 7.65 ppm, respectively.

After treatment of fresh clean castor bean leaves with each of **LC_{50} values** of the previously mentioned CSIs, newly moulted last instar larvae were starved for 4 hrs and enforced (No-choice) to feed on the treated plant leaves for 24 hrs or 72 hrs, then provided with untreated clean plant leaves. Control last instar larvae were provided with untreated leaves. Ten larvae were used as replicates for each treatment and control. The replicates were kept individually in 250 ml glass jars for observing and determining the nutritional parameters as described herein.

3. Antifeedant activity:

Antifeedant index (AFI %) was calculated according to the equation of [82] as follows: AFI % = [(C-T)/(C +T)] x 100 Where C: amount of food eaten by the control insect. T: amount of food eaten by the treated insect.

4. Efficiencies of Food Metabolism:

In the present work, food consumption, digestion, absorption and conversion efficiencies were determined on a daily basis along the last larval instar of *S. littoralis*. Body weight of both treated and control was recorded before and after feeding, fresh food leaves were weighed before introduction to the larva, and then the fresh weight of remains was recorded after feeding every day. For calculating the corrected weight of consumed food, known weights of fresh food leaves were left without larva for 24 h, under the same laboratory conditions, and re-weighed at the end of this interval. Weight of faeces is the amount of frass produced by the larva during the last instar.

Relative weight gain (RWG) = mg weight gain during the instar/ days [83] with correction for a single instar.

Feeding rate is the amount of food consumed per instar along its feeding period; generally expressed on a "per day per unit body mass" basis [84]. **Relative consumption rate** was calculated according to [85] as follows: RCR = mg consumed food/ (g mean fresh body weight/ day).

According to [86], the following parameters can be calculated. Approximate digestibility (AD) = [(Weight of ingested food - Weight of faeces)/ Weight of ingested food] X 100. Efficiency of conversion of ingested food to body substance (ECI) = [Weight gain / Weight of ingested food] X 100. Efficiency of conversion of digested food to body substance (ECD): [Weight gain / (Weight of ingested food - Weight of faeces)] X 100.

Assimilation rate (AR) = RCR x AD [61].

Relative metabolic rate (RMR) was calculated according to [87] but corrected for fresh weights and for a single nymphal instar as follows:

RMR = (mg weight ingested food - weight of faeces) / (g mean fresh body weight / day).

These parameters may help to clear the metabolic efficiencies which can affect growth [83,88]. **Growth rate** (GR) can be calculated as follows: GR = fresh weight gain during feeding period / (feeding period X mean fresh body weight of larvae during the feeding period) [86].

5. Statistical analysis of data:

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [89] for the test significance of difference between means.

RESULTS

1. Antifeedant activities of CSIs against *S. littoralis* larvae:

Data arranged in Table (**1**) clearly show a considerable antifeedant activity of each of all tested CSIs, *viz*. Novaluron (Nova.), Cyromazine (Cyro.) and Diofenolan (Dio.), which increased by the longer feeding period of last instar larvae of *S. littoralis*. For some detail, feeding of larvae on castor bean leaves treated with these CSIs for 72 hrs resulted in food deterrence (37.9, 28.44 & 34.25%, by Nova., Cyro. and Dio., respectively) higher than that recorded after feeding for 24 hrs (10.33, 7.16 & 9.48%). Whatever the feeding period, **Nova.** exhibited the strongest antifeedant activity followed by Dio. and Cyro..

2. Effects of CSIs on food ingestion and consumption of *S. littoralis* larvae:

Depending on the data distributed in Table (**2**), feeding of larvae on treated leaves for **24 hrs** resulted in remarkable **reduction** of food intake. The strongest reducing effect was exhibited by **Nova.** followed by Dio. and Cyro. (237.18±18.16, 277.93±25.11and 291.21±21.36 mg after feeding on leaves treated with Nova., Dio. and Cyro., respectively, *vs*. 336.13±12.57 mg food consumed by control larvae). As expressed in the relative consumption rate (RCR), the drastically regressed rate was recorded for larvae fed on **Nova.** but other CSIs exerted insignificantly regressing actions on such rate.

After feeding on treated leaves for **72 hrs**, food intake was considerably reduced, regardless the CSI. However, the strongest reducing effect was exhibited by **Nova.** (151.36±17.30 mg) followed by Dio. (164.63±13.33mg) and Cyro. (187.27±9.88 mg, compared to 336.13±12.57 mg consumed by control larvae). Also, RCR was dramatically suppressed proportionally to the reduced food consumption (Table **3**).

As exiguously shown in Tables **(2**&**3)**, feeding of larvae on Nova.-treated leaves for **72 hrs** resulted in higher inhibition of food consumption than feeding for **24 hrs** (Change %s: -75.16, compared to -51.93). A similar trend had not been observed after treatment with Cyro. or Dio. since change %s were calculated as -48.96 and -48.62 after feeding on leaves treated with these two CSIs for 24 hrs, respectively, but as -42.04 and -46.50 after feeding for **72 hrs**. However, data of food intake or RCR clearly show stronger reducing effects of CSIs had been exhibited by longer feeding period.

3. Effects of CSIs on food digestive, absorptive and conversion efficiencies of *S. littoralis* larvae:

According to data presented in Table (**4**), larvae achieved significantly **inhibited** approximate digestibility (**AD**) as a result to feeding on CSI-treated leaves. **Nova.** exhibited the most potent effect followed by Dio. and Cyro. (Reductions: 26.11, 16.22 & 15.65%, by these CSIs, respectively).In addition, AD values of larvae were elaborately lower after feeding on CSI-treated leaves for **72 hrs**, as obviously observed in Table (**5**). Reductions were determined in70.82, 48.09 and 47.86% after treatment with Nova., Dio. and Cyro., respectively. On comparing data of Tables (**4&5**), longer feeding period on CSI-treated leaves resulted in larger stress on larvae to attain weaker AD capacity.

In the light of data assorted in Table (**4**), efficiency of conversion of ingested food into biomass (**ECI**) severely affected by the tested CSIs since it was found in pronouncedly **decreased** values (44.52±2.74, 43.33±3.12 and 48.33±2.18%, after treatment with Nova., Cyro. and Dio., respectively. Depending on the calculated change%, the **greatest reduction** of ECI was caused by **Cyro.** followed by Nova. and Dio. (-70.00, -14.25 and -6.91, respectively). For investigating the affected ECI by feeding on treated food for **72 hrs**, data presented in Table (**5**) clearly revealed a slightly **reduced** ECI by Cyro. (3.68% decrease) or Cyro. (9.84% decrease). Unexpectedly, Nova. exhibited an inverse effect because it **promoted** ECI of larvae (60.23±7.33 *vs*. 51.92±2.10 of control larvae) as an exceptional case. Thus, reducing effect of the tested CSIs on ECI of larvae after feeding on treated food for **24 hrs** was stronger than that exhibited by feeding for **72 hrs**.

On the basis of data arranged in Table (**4**), efficiency of conversion of digested food into biomass (**ECD**) of last instar larvae was considerably **enhanced** (74.12±4.06, 70.51±3.56 and 70.96±3.33, after treatment with Nova., Cyro. and Dio., respectively, for **24 hrs**, compared to 63.86±2.33 of control larvae). Thus, the most potent CSI was Nova. followed by Dio. and Cyro.. **In contrast**, feeding of larvae for **72 hrs** resulted in serious **reduction** of **ECD** (reduction %s: 53.81, 48.01 and 41.67, after treatment with Nova., Cyro. and Dio., respectively, Table **5**). As seen in these data, **Nova.** exhibited the strongest

reducing effect on ECD of larvae after feeding for **72 hrs**.

4. Effects of CSIs on the food assimilation by*S. littoralis* larvae:

For extensive investigation of the food metabolism, two additional metabolic parameters (assimilation rate, AR and relative metabolic rate, RMR) may shed some light on the effects of CSIs. As obviously shown in Table (**6**), AR was significantly **regressed** by all CSIs and the **most suppressing action** was exerted by **Nova.** after feeding for 24 hrs (52.26±2.05, compared to 147.15±5.13% of control congeners). Similar suppressing action was exerted also by **Nova.** after feeding for 72 hrs (9.25±1.03 *vs*. 147.15±5.13% of control congeners). However, the longer feeding period led to stronger reducing effects of CSI on the assimilation capacity of larvae (Table **7**). Just a look at data of tables (**6&7**) revealed similar inhibitory effects of CSIs on RMR of larvae and the superior inhibitory compound was **Nova.**

Recalling data arranged in tables 2, 3, 4, 5, 6&7shows a positive correlation of AR and RMR to RCR, AD and ECI which was easily detected because these parameters were reduced by all tested CSIs, indicating a prohibited capacity of larvae to digest, absorb and assimilate the food eaten at lower RCR.

5. Effects of CSIs on somatic growth and frass production by S*. littoralis* larvae:

After feeding of larvae on plant leaves treated with CSIs for **24 hrs**, data of relative weight gain (RWG), growth rate (GR) and frass production had been summarized in Table (**6**). RWG was drastically regressed as response to all tested CSIs and the **least RWG** was recorded after treatment with **Dio.** (20.32±0.41 mg *vs*. 29.09±0.67 mg of control congeners). GR was also rigorously declined, regardless the CSI, and the least GR was recorded after treatment with **Nova.** (6.97±0.11, compared to 13.33±0.76 of control larvae). As shown in Table (**7**), stronger inhibitory effects of CSIs on RWG and GR had been exhibited after feeding of larvae on treated food for **72 hrs**. Also, **Dio.** was found as the strongest reducing compound on RWG (13.72±0.67 *vs*. 29.09±0.67 of control larvae) and **Nova.** Exhibited the strongest reducing effect on GR (5.07±0.67 *vs*. 13.33±0.76 of control larvae).

On the contrary, frass output was enhanced by all tested CSIs because treated larvae excreted **increasing amounts of fecal pellets** whatever the feeding period on treated leaves (Tables **6**&**7**). Moreover, **Nova.** was found as the most powerful CSI to promote larvae for frass production (109.33±6.64 and 115.46±13.25 mg, after feeding for 24 hrs and 72 hrs, respectively). Thoroughly looking at data of these tables shows that larger amounts of frass had been produced by larvae after longer feeding period on CSI-treated food.

Table 1: Antifeedant activity (%) of CSIs (LC_{50}) against last instar larvae of *S. littoralis*.

Feeding period (hr)	CSI			Control
	Novaluron	Cyromazine	Diofenolan	
24	10.33	7.16	9.48	---
72	37.9	28.44	34.25	---

Table (2): Food ingestion and consumption of last instar larvae of *S. littoralis* after feeding on fresh castor bean leaves treated with LC_{50} values of CSIs for 24hrs.

CSI	Food intake (Mean mg±SD)	RCR (Mean±SD)	Change (%)
Novaluron	273.18±18.16 d	1.22±0.11 b	-51.93
Cyromazine	291.21±21.36 d	1.55±0.09 a	-48.96
Diofenolan	277.93±25.11 d	1.55±0.13 a	-48.62
Control	336.13±12.57	1.57±0.39	---

SCI: chitin synthesis inhibitor. RCR: relative consumption rate of food. Mean ± SD followed with (a): insignificantly different (P >0.05), (b): significantly different (P<0.05), (d): very highly significantly different (P<0.001).

Table 3: Food ingestion and consumption of last instar larvae of *S. littoralis* after feeding on fresh castor bean leaves treated with LC_{50} values of CSIs for 72hrs

CSI	Food intake (Mean mg±SD)	RCR (Mean±SD)	Change (%)
Novaluron	151.36±17.30 d	0.39±0.03 d	-75.16
Cyromazine	187.27±9.88 d	0.91±0.08 c	-42.04
Diofenolan	164.63±13.33 d	0.84±0.03 c	-46.50
Control	336.13±12.57	1.57±0.39	---

CSI, RCR, d: See footnote of Table (2). (c): highly significantly different (P<0.01).

Table 4: Food digestion, absorption and utilization of last instar larvae of *S. littoralis* after feeding on fresh castor bean leaves treated with LC_{50} values of CSIs for 24hrs.

CSI	AD (Mean±SD)	Change (%)	ECI (Mean±SD)	Change (%)	ECD (Mean±SD)	Change (%)
Novaluron	60.07±3.17 d	- 26.11	44.52±2.74 d	- 14.25	74.12±4.06 d	+16.07
Cyromazine	68.58±2.95 d	- 15.65	43.33±3.12 d	- 70.00	70.51±3.56 a	+ 11.10
Diofenolan	68.11±2.07 d	- 16.22	48.33±2.18 c	- 6.91	70.96±3.33 d	+11.12
Control	81.30±1.98	---	51.92±2.10	---	63.86±2.33	---

CSI, a, d: See footnote of Table (2). AD: Approximate digestibility, ECI: Efficiency of conversion of ingested food, ECD: Efficiency of conversion of digested food. c: See footnote of Table (3).

Table 5: Food digestion, absorption and utilization of last instar larvae of *S. littoralis* after feeding on fresh castor bean leaves treated with LC_{50} values of CSIs for 72hrs.

CSI	AD (Mean±SD)	Change (%)	ECI (Mean±SD)	Change (%)	ECD (Mean±SD)	Change (%)
Novaluron	23.72±4.16 d	- 70.82	60.23±7.33 c	+16.01	46.19±6.67 d	- 53.81
Cyromazine	42.39±8.33 d	- 47.86	46.81±5.17 b	- 9.84	51.94±5.18 d	- 48.01
Diofenolan	42.20±6.67 d	- 48.09	50.01±6.67 a	- 3.68	58.33±4.67 b	- 41.67
Control	81.30±1.98	---	51.92±2.10	---	63.86±2.33	---

CSI, a, d: See footnote of Table (2). AD: Approximate digestibility, ECI: Efficiency of conversion of ingested food, ECD: Efficiency of conversion of digested food. c: See footnote of Table (3).

Table 6: The correlation of AR and RMR to RWG and GR of *S. littoralis* after feeding on fresh castor bean leaves treated with LC_{50} values of CSIs for 24hrs.

CSI	AR (Mean±SD)	RMR (Mean±SD)	RWG (Mean±SD)	GR (Mean±SD)	Faeces output (Mean mg±SD)
Novaluron	52.26±2.05 d	1.22±0.14 d	22.39±0.17 d	6.97±0.11 d	109.33±6.64 d
Cyromazine	65.84±3.17 d	1.58±0.17 a	21.03±0.33 d	8.61±0.82 d	91.50±9.07 d
Diofenolan	63.34±1.48 d	1.55±0.13 a	20.32±0.41 d	9.15±0.33 d	88.63±7.13 d
Control	147.15±5.13	1.57±0.13	29.09±0.67	13.33±0.76	62.87±6.74

CSI, a, and d: See footnote of Table (2). AR: Assimilation rate (x 100), RMR: Relative metabolic rate (x 100), RWG: Relative weight gain, GR: Growth rate (x100).

Table 7: The correlation of AR and RMR to RWG and GR of *S. littoralis* after feeding on fresh castor bean leaves treated with LC_{50} values of CSIs for 72hrs.

CSI	AR (Mean±SD)	RMR (Mean±SD)	RWG (Mean±SD)	GR (Mean±SD)	Faeces output (Mean mg±SD)
Novaluron	9.25±1.03 d	0.39±0.05 d	15.19±1.33 d	5.07±0.67 d	115.46±13.25 d
Cyromazine	38.57±2.33 d	0.95±0.03 d	14.61±0.92 d	6.15±0.77 d	107.88±9.25 d
Diofenolan	35.45±3.67 d	0.86±0.01 d	13.72±0.67 d	7.27±0.39 d	95.16±6.14 d
Control	147.15±5.13	1.57±0.13	29.09±0.67	13.33±0.76	62.87±6.74

CSI, d: See footnote of Table (2). AR, RMR, RWG, and GR: See footnote of Table 5.

Depending on the data listed in Tables 2, 3, 4, 5, 6 & 7, reduction of RWG and GR had been found in a positive correlation to the inhibition of AD and ECI, regardless the feeding period. On the other hand, increasing fecal production was reversely correlated to decreasing RCR of treated larvae.

DISCUSSION

Food utilization efficiencies are useful for measuring the growth rate and development of the consumer [61]. Several metabolic parameters were suggested and usually used to determine the food utilization. However, the common three efficiencies are: approximate digestibility (AD), efficiency of conversion of ingested food to biomass (ECI) and efficiency of conversion of digested food to biomass (ECD) [86,90,84]. As described by [91], ECI is an overall measure of an insect's ability to utilize the ingested food for growth and development and ECD is a measure of the efficiency of conversion of digested food into growth. ECD is sometimes called "Net growth efficiency" or "Metabolic efficiency" [60].ECI and ECD vary widely with the insect species. As for example, ECI and ECD of lepidopterous larvae are about double those of orthopterous larvae, while AD being about the same. The efficiencies of food utilization also vary with age (both within and between instars) and sex as well as with different environmental factors.

1. Antifeedant activity of Chitin synthesis inhibitors (CSIs) against *S. littoralis* larvae:

Some authors did not determine the food deterrence or antifeedant activity of insecticides or IGRs against the target insect pests but used

the food consumption as a good indicator for it. On the other hand, few researchers recorded antifeedant index or deterrence index. As reported in the available literature, osthole and pregnenolone exhibited significant antifeedant activity against *Spodoptera litura* larvae [92]. [93] reported that α-phellandrene- and β-ionone exhibited the strongest deterrent effect against *Pieris brassicae* 4th instar larvae but (S)-(+)-carvone exhibited a slight antifeedant effect. [94] recorded an antifeedant activity of chlorpyrifos and deltamethrin, individually and in combination, on the *Atractomorpha crenulata* 4th instar nymphs. With special reference to *Spodoptera littoralis*, chlorantraniliprole, thiamethoxam and novaluron exhibited feeding deterrent action against 4th instar larvae [95]. On feeding of the 4th instar larvae on castor bean leaves treated with emamectin benzoate, rynaxypyr, indoxacarb, spinetorm or spinosad for 24 hrs, the highest inhibition of feeding and antifeedant index were recorded for indoxacarb and rynaxypyr [96].

In the present study, all tested CSIs, *viz*. Novaluron, Cyromazine and Diofenolan, exhibited considerably antifeedant activities against the last instar larvae of *S. littoralis*. Higher antifeedant activity was exhibited after the longer feeding period. Whatever the feeding period, 24 or 72 hrs, Novaluron exhibited the strongest antifeedant activity followed by Diofenolan and Cyromazine. To understand the antifeedant activity of the tested CSIs, they may stimulate specific 'deterrent' cells in chemoreceptors and also block the firing of 'sugar' receptor cells, which normally stimulate feeding [97,98]. These results in the feeding inhibition, culminating in the starvation and death of the insect by feeding deterrence alone [99].

2. Food consumption by *S. littoralis* larvae as influenced by CSIs:

Depending on the reported results in the available literature, food consumption had been significantly reduced in several insect species by various insecticides or IGRs and IGR-related compounds. A considerable reduction in the food consumption was determined for *Leptinotarsa decemlineata* larvae by Flucycloxuron [100], for *S. gregaria* adults by fenitrothion [101], and for *Callosobruchus muculatus* larvae by Cyromazine [102]. With regard to *S. littoralis*, significantly reduced food consumption of larvae was observed after feeding on castor bean leaves treated with Mancozeb, bromoxynil and profenofos [103], Pyriban (Chlorpyrifos) [104], chlorantraniliprole, thiamethoxam and novaluron [95], rynaxypyr and indoxacarb [96], Flufenoxuron and triflumuron [105], Diazinon and flufenoxuron [106] or chlorfenapyr [107].

Results of the present study are in agreement with those reported results since feeding of *S. littoralis* last instar larvae on treated leaves for **24 hrs** or **72 hrs** resulted in remarkable **reduction** of food intake, regardless the CSI. The strongest reducing effect was exhibited by **Novaluron** followed by **Diofenolan** and **Cyromazine**. Longer feeding period on **Novaluron**-treated leaves led to higher reduction of food consumption. On the other hand, the present results disagree with the reported results of increasing food consumption by some insects after treatment with some chemicals, such as *Spodoptera mauritia* last instar larvae after treatment with Hydroprene [108] and Spanish slug *Arion lusitanicus* adults after treatment with deltamethrin and pyriproxyfen [109]. However, the remarkable reduction of food consumption of *S. littoralis* last instar larvae by the tested CSIs, in the present study, can be attributed to their direct or indirect interferences with the hormonal regulation of food intake [110]. It can be interpreted, also, by the partial avoidance of *S. littoralis* larvae to introduce food after treatment with these CSIs or they adversely affected the mandibles and labrum or blocked the gut function [111].

3. Food digestive and absorptive capacities of *S. littoralis* larvae as influenced by CSIs:

Special attention should be paid to another important nutritional parameter, AD, which expresses the digestion and absorption capacity of the insect. AD in insects is based on differences between the weight of ingested food and the weight of faeces, actually represents the food which is stored or metabolized. Therefore, the AD estimates the percentage of ingested food that is digested [60].

In the present study, *S. littoralis* larvae achieved significantly **inhibited AD** as a response to feeding on CSI-treated leaves. **Novaluron** exhibited the most potent effect followed by Diofenolan and Cyromazine. Longer feeding period on treated leaves resulted in weaker AD capacity. These results are, to a great extent, in accordance with those results of reduced AD of some insects by different IGRs and other chemicals since significantly reduced AD was recorded for *S. gregaria* nymphs after treatment with Fenoxycarb [112] and for *S. littoralis* larvae after treatment with Fenoxycarb [77], Teflubenzuron [113], Sumialfa [114]or rynaxypyr and indoxacarb [96]. In the same

lepidopteran, AD was inhibited in the 4th instar larvae after treatment with LC_{50} of some **IGRs** [115] and in the 6th instar larvae after treatment with **Cadmium** [116]. The inhibited AD of *S. littoralis* last instar larvae, **in the present study**, may be due to the toxicity of the tested CSIs leading to damage of the digestive and absorptive cells of gut epithelium, thereby may impair the food digestion and absorption capacity as suggested, also, for *Helicoverpa armigera* [117] and *S. littoralis* [116] after treatment with the heavy metal cadmium. In additio the food digestion and absorption through the damage of peritrophic membrane [118].

4. Food conversion efficiencies of *S. littoralis* larvae as influenced by CSIs:

From the metabolic view of point, the most important efficiencies of food conversion into biomass are ECI and ECD. According to the results reported in literature, **ECI** of various insect species had been considerably or slightly reduced by different insecticides and IGRs, such as fenarimol [77], tebufenozide [79], Sumialfa [114], flufenoxuron and triflumuron [105], rynaxypyr and indoxacarb [96], Diazinon and flufenoxuron [106] or chlorfenapyr [107] against *S. littoralis* larvae. In concomitant with those reported results, the current work on larvae of the same lepidopterous insect pest revealed remarkable **inhibition of ECI** by the tested CSIs, regardless the feeding period. The strongest reducing effect was exhibited by Cyromazine followed by Novaluron and Diofenolan. On the other hand, an exceptional case of **enhancement** of **ECI** was recorded for Novaluron after feeding of larvae on treated leaves for 72 hrs. This case agrees, to some extent, with the reported results of significantly **increasing** ECI in the last instar larvae of *S. mauritia* by Hydroprene [108] and in the last instar larvae of *S. littoralis* by Cadmium [116].

With regard to **ECD** of *S. littoralis* in the present study, feeding of last instar larvae on CSI-treated leaves for 72hrs resulted in serious **reduction** of this nutritional parameter. Novaluron exhibited the strongest **reducing** effect on ECD of larvae. The current results are in congruence with several reported results of reduced ECD of *S. littoralis* larvae by different insecticides and IGRs, such as Sumialfa [114], flufenoxuron and triflumuron [105], rynaxypyr and indoxacarb [96], Diazinon and flufenoxuron [106], chlorfenapyr [107], etc. **In contrast**, feeding of *S. littoralis* larvae on CSI-treated leaves for 24 hrs, **in the present study**, resulted in considerably **increased** ECD. The most potent **enhancing** CSI was Novaluron followed by Diofenolan and Cyromazine.

In this respect, [119] reported that ECI will vary with the digestibility of food and proportional amount of the digestible portion of food which is converted to body substance and metabolized for energy to maintain life. However, the **reduction of ECI and ECD** of *S. littoralis* larvae, in **the current investigation**, may indicate more food is being metabolized for energy purpose and less for conversion to biomass [120]. On the other hand, the **induced ECI** of larvae of this insect may be attributed to the fact that they require large amounts of energy to deal with the used CSIs toxicities, as suggested by [121] for *Galleria mellonella* and [117] for *H. armigera*. Unfortunately, we have no conceivable interpretation to the **increased ECD** of *S. littoralis* larvae after treatment with the tested CSIs right now!!

5. Food assimilation and metabolism in *S. littoralis* larvae as influenced by CSIs:

Some other nutritional parameters had been interestingly used in this area of study, *viz*. Assimilation rate (AR) and Relative metabolic rate (RMR). These parameters may help to clear the metabolic efficiencies which can affect the growth [88].**In the present study**, **AR** of *S. littoralis* larvae was significantly **regressed** by all tested CSIs. The **most suppressing action** was exerted by **Novaluron**. However, the longer feeding period potentiated stronger reducing effects on the assimilation capacity of larvae. Similar inhibitory effects of tested CSIs on RMR of *S. littoralis* larvae had been recorded and the superior inhibitory one was **Novaluron**. These results are, to some extent, in agreement with those reported results of regressed AR and RMR in larvae of various insect species by the action of some IGRs, such as *Agrotis ipsilon* [122], *Manduca sexta* [123], *S. litura* [124], *S. littoralis* [79] and *S. gregaria* [112].

6. Interrelationship between growth and nutritional performance of *S. littoralis* larvae under stress of CSIs:

As clearly reported in the literature, relative weight gain **(RWG)** or/and relative growth rate **(RGR)** of many insects had been declined by several insecticides or IGRs and IGR-related compounds. Concerning *S. littoralis*, RGR of larvae was significantly reduced by some IGRs [115], chlorantraniliprole, thiamethoxam and Novaluron [95], flufenoxuron and Triflumuron [105] or rynaxypyr and indoxacarb [96]. Also, feeding of the 2nd instar larvae of *S. littoralis* on plant leaves treated with Diazinon and

flufenoxuron resulted in reduction of RGR [106]. In *A. crenulata*, Chlorpyrifos showed stronger growth inhibitory action than deltamethrin [94]. To a great extent, results of the present study agree with those reported results since feeding of *S. littoralis* larvae on plant leaves treated with Novaluron, Cyromazine or Diofenolan, for **24 hrs** or **72 hrs**, resulted in drastically regressed RWG. The **least RWG** was recorded after treatment with **Diofenolan**. The growth rate (GR) was also rigorously declined, regardless the CSI. The **least GR** was recorded after treatment with **Novaluron**. The tested CSIs exhibited stronger inhibitory effects on RWG and GR of larvae by longer feeding period on treated food. However, reduction of RWG and inhibition of GR of *S. littoralis* larvae after treatment with the tested CSIs may be due to the reduction of food consumption. This suggestion may be substantiated by similar reduction of larval growth of the same lepidopteran by cadmium [116]. Also, the growth inhibition may be attributed to the disruptive effects of the tested CSIs on the peritrophic membrane of the midgut [125] or to the use of food for purposes other than growth, such as detoxification enzymes synthesis [126].

With regard to the **fecal production** by larvae, it is important to point out that feeding is necessary for the stimulation of digestive enzyme activities [127] and may have interfered with the enzyme-substrate complex thus affecting the peristaltic movement of the gut [128]. Some IGRs prohibited the fecal production by insects since *S. littoralis* larvae produced remarkably reduced faeces after treatment with fenarimol or naurimol [77], tebufenozide [79] or Lufenuron [129]. Also, reduction of fecal production was recorded for *S. gregaria* after treatment with fenoxycarb [112] and for *S. mauritia* after treatment with diflubenzuron [118]. Dissimilar to those reported results, the present results showed **induced** frass production by last instar larvae of *S. littoralis* as response to all CSIs, regardless the feeding period on treated leaves. Moreover, **Novaluron** was found as the most powerful CSI to promote larvae for the frass production. Whereas interpretation of the reduction of frass production by some insects as response to action of various IGRs or insecticides was provided by same authors [128,130], we are unable right now to provide a conceivable explanation to the **increasing fecal production** by *S. littoralis* larvae after treatment with the tested CSIs. Likewise, these increased fecal pellets may contain undigested food remains since AD was significantly suppressed.

CONCLUSION

As obviously shown in the present study, **Novaluron**, **Cyromazine** and **Diofenolan** exhibited considerably antifeedant activities against *S. littoralis* last instar larvae. These CSIs remarkably **reduced** the food consumption and adversely inhibited the approximate digestibility, efficiency of conversion of ingested food into biomass, efficiency of conversion of digested food into biomass and other parameters of food metabolism, with few exceptions. Therefore, the tested CSIs can be considered as promising agents for controlling the dangerous phytophagous pest *S. littoralis*, especially Novaluron and Diofenolan.

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